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CERTAIN VARIATIONS IN THE MORPHOLOGY OF TUBERCLE  
BACILLI OF BOVINE ORIGIN.

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## CERTAIN VARIATIONS IN THE MORPHOLOGY OF TUBERCLE BACILLI OF BOVINE ORIGIN.

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The wide variation in morphology of the tubercle bacillus is strikingly shown in the recent work of Wolbach and Ernst,<sup>1a</sup> which consists in a careful study of the variations in form of the human and bovine bacillus at different ages and on different media. Perhaps the most striking variation noted by these authors occurred in the case of the cultures grown on brain medium, from which long, thread-like, and branched forms were obtained, the human bacilli ranging from 7 to 14  $\mu$  and the bovine from 6 to 8  $\mu$  in length. These long, thread-like forms are described as staining irregularly and as being made up of alternate faint and deeply stained areas, and some excellent photomicrographs are shown in which the curiously segmented appearance of the threads is well brought out. As the foregoing is the first instance in which long, thread-like forms have been reported for the bovine bacillus, the following observations may be of interest.

In examining some old cultures of *Bacillus tuberculosis*, grown on Dorset's egg medium,<sup>2</sup> it was noticed in several instances that the paraffin used in sealing the tubes had run down and across the surface of the egg, and in each case there appeared along the edges of the streak of paraffin an increased growth or a piling up of the tubercle bacillus, which seemed to be actually encroaching on or growing over the surface of the paraffin. There were three tubes in which this had occurred, namely, cultures of bovine, swine, and dog tuberculosis, which had been cultivated for a number of generations on bouillon before being transferred to egg. The age of these cultures was twenty-one weeks, but the length of time the paraffin had been in contact with the growth was not known, as the cultures had been set aside and neglected for some time. To the naked eye the growths bordering the paraffin were white in color and they separated readily from the paraffin in large masses. Cover-slip preparations from these growths, stained with carbol-fuchsin and decolorized with 25 per cent  $H_2SO_4$ , revealed in each case long thread-like, beaded forms, measuring from 6 to 8  $\mu$  in length, the bovine and swine reaching 8  $\mu$  in length (Pl. IV, figs. 1 and 2). It will be noticed that the threads show alternate stained

<sup>a</sup>These numbers refer to the bibliography at the end of the article.

and faintly stained areas, presenting a beaded appearance very similar to the forms described by Wolbach and Ernst for the brain medium.

Culture tubes of egg, prepared by flowing sterile paraffin (melting point 43° C.) over the surface so as to form a streak, as in the tubes noted above, were inoculated with some of the normal growth—that is, growth away from the paraffin—from the original bovine and swine cultures from which the long, thread-like forms were first obtained, and another from a freshly isolated bovine culture marked Heifer No. 213, the surface of the egg near the paraffin streak being inoculated in each case. These cultures showed the same piling up of the growth along the margin of the paraffin as in the original tubes, and at twenty weeks the growth in all these tubes had coalesced at the center, completely bridging over the streak of paraffin, which averaged about 5 mm. in width. Inoculations on plain egg without the addition of paraffin were made at the same time for the sake of comparison, and preparations from these and the foregoing cultures were measured with the following results, the cover-slip preparations being made always from the inner edge of the growth covering the paraffin in the case of those tubes to which the paraffin was added:

*Tubercle bacilli grown on egg in contact with paraffin and on plain egg.*

Number of culture.	Egg and paraffin.			Plain egg.		
	2 weeks.	6 weeks.	17 weeks.	2 weeks.	6 weeks.	17 weeks.
	Microns.	Microns.	Microns.	Microns.	Microns.	Microns.
Swine I, 36 .....	2.5	3	8	2	2.5	3
Bovine III, 37 .....	2	2	3	2	2	2
Heifer No. 213 .....	2	2	3.5	2	2	2

The measurements were made with an ocular micrometer and represent an average of the longest forms in each preparation measured.

It will be noticed in this case that of the cultures to which paraffin had been added, Swine I was the only one that showed marked lengthening, though both of the other cultures showed a tendency to lengthen out, whereas no such tendency was apparent in the case of the cultures grown on plain egg. The growths in contact with the paraffin all showed beading at seventeen weeks.

A culture tube of egg, prepared by flowing sterile paraffin across the surface, was also inoculated from the inner or encroaching edge of the growth bordering the paraffin in the original bovine culture<sup>a</sup> from which the long, filamentous forms were first obtained. This culture, labeled B<sub>1</sub>, showed the same piling up of the growth along the edge of

<sup>a</sup>This culture had been cultivated for thirty-seven generations on bouillon prior to its transfer to egg, and the individual organisms at the time of its transference from the bouillon averaged 2.5  $\mu$  in length.

the paraffin streak, and from it, when eighteen weeks old, a second tube, prepared in the same way, was inoculated in the same manner—that is, from the inner edge of the growth bordering the paraffin, and labeled B<sub>2</sub>. Measurements of these cultures gave the following results:

*Bovine bacillus grown on egg in contact with paraffin.*

Culture.	2 weeks.	6 weeks.	17 weeks.	Check at 17 weeks.
	Microns.	Microns.	Microns.	Microns.
B <sub>1</sub> .....	2	2.5	8	2
B <sub>2</sub> .....	2	3	5	2

From these last measurements it will be seen that growth in contact with the paraffin does not tend to impart any fixed characteristic to the bovine bacillus; that is, the long forms are not perpetuated from one generation to the next.

Inoculations on plain egg without the addition of paraffin were also made from the inner or encroaching edge of the growth bordering the paraffin in the original tubes, from which the long, thread-like forms were first obtained, but in each instance they grew out in the usual short forms characteristic of the egg medium, without any apparent tendency to lengthen. Drawings made with the camera lucida from the bovine culture B<sub>1</sub>, grown on egg in contact with paraffin and on plain egg, are shown in Plate V. It is interesting to note that no branching was observed in the filamentous forms obtained from the growths bordering the paraffin.

Cultures of *Bacillus tuberculosis* obtained from different sources, both human and bovine, inoculated on egg across the surface of which sterile paraffin has been flowed to form a streak, show in every case an increase or piling up of the growth along the edges of the paraffin. The organism appears, in fact, to possess a decided affinity for the paraffin, manifesting itself earlier and progressing more rapidly where it comes in contact with the paraffin. In old cultures the growth often completely covers the paraffin, and in some instances where the paraffin is piled up at the bottom of the tubes in large, rounded masses the growth has completely enveloped these masses and continues to grow over the surface of the paraffin after the growth on the surrounding medium has ceased.

Culture tubes of blood serum, glycerine agar, and plain neutral agar, treated with paraffin, were also inoculated with human and bovine tubercle bacilli and the same increase or piling up of the growth was noted at the edges of the paraffin streak, although the increase in growth was not so striking as in the cultures grown on egg. Cover-slip preparations from the growths bordering the paraffin show, in

most cases, that the individual organisms tend to lengthen out after a period of six to seventeen weeks; this tendency, however, does not appear to be constant.

The points of more especial interest in the preceding observations are the occurrence of the long, filamentous forms in connection with the bovine bacillus, and the increased growth in contact with the paraffin.

Filamentous and branched forms of the tubercle bacillus have been frequently noted in avian cultures, in old human cultures, and in cultures that have been subjected to abnormal conditions of growth, such as unfavorable temperatures or media; but until the recent work of Wolbach and Ernst<sup>1</sup> no such forms had been reported in the case of the bovine bacillus. The bovine bacillus, in fact, has always been regarded as more stable in its morphology and less liable to changes in form than the other varieties, so called, of the tubercle bacillus. Thus Theobald Smith,<sup>3</sup> in summarizing his observations on the morphological and biological characters of human and bovine bacilli, states that "bovine bacilli are much less influenced by certain modifications of the culture medium" and "tend to remain short; human bacilli are either more slender from the start or become so during cultivation." A comparison, however, of the extremely short forms characteristic of Dorset's egg medium with the long, thread-like forms noted for the egg in contact with paraffin, and for the brain medium, would seem to show that modifications of the culture medium have a marked effect on the bovine as well as on the human bacillus, and that the bovine bacillus, like the human bacillus, is subject to wide variation in morphology, and that constancy of form under cultivation can hardly be regarded as a distinctive characteristic of the bovine bacillus.

Another point of interest in connection with the growths noted in contact with the paraffin is the occurrence of forms representing the two extremes in morphology on the same medium and in the same culture, the long filamentous forms where the growth borders on the paraffin, and the short micrococcus-like forms from the surface of the egg.

Inasmuch as paraffin is a very stable and indifferent chemical substance, an explanation of the increased growth in contact with this substance would seem to rest on a purely physical basis. An explanation that suggests itself is that the paraffin, cooling suddenly in contact with the egg, incloses a considerable quantity of air in its interstices and thus supplies the growing organism with oxygen. This would account for the continued growth at the margins and over the surface of the paraffin after the growth has ceased on the surface of the egg; it would also account for the continued growth over the paraffin even after the cotton plugs (sealed with paraffin) have been drawn down, in consequence of the exhaustion of the oxygen of the air contained within the tube and the resulting partial vacuum. Furthermore, it might explain



FIG. 1.—BOVINE TUBERCULOSIS, TWENTY-ONE WEEKS ON  
EGG MEDIUM PLUS PARAFFIN. X1400.

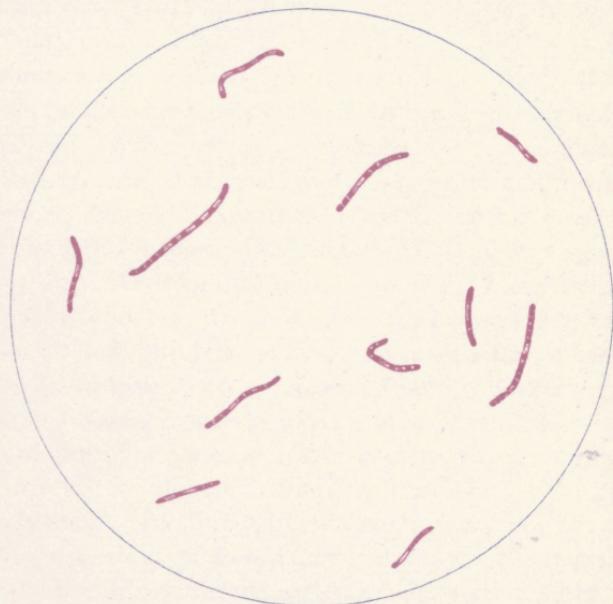


FIG. 2.—SWINE TUBERCULOSIS, TWENTY-ONE WEEKS ON  
EGG MEDIUM PLUS PARAFFIN. X1400.

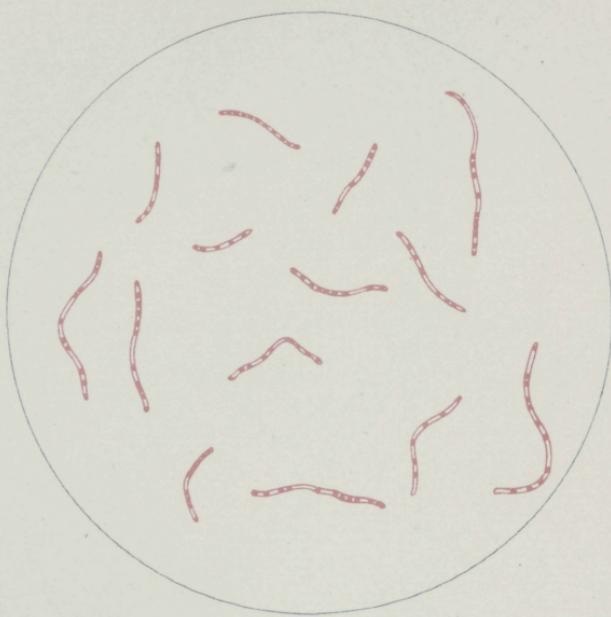


FIG. 1.— BOVINE TUBERCULOSIS, SEVENTEEN WEEKS ON  
EGG MEDIUM PLUS PARAFFIN. X1400.



FIG. 2.— BOVINE TUBERCULOSIS, SEVENTEEN WEEKS ON  
PLAIN EGG MEDIUM. X1400.

the occurrence of the filamentous forms in contact with the paraffin; for, according to Coppen-Jones,<sup>4</sup> the conditions best suited for the production of filamentous and branched forms are a favorable medium and an abundance of oxygen. No doubt the paraffin also protects the surface of the egg from evaporation and consequent drying and thus preserves the moisture so essential to the growth of the tubercle bacillus. It must be confessed, however, that this explanation does not appear wholly satisfactory, but none other has suggested itself.

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